Animal Reproduction

Abstracts - 35th Annual Meeting of the Brazilian Embryo Technology Society (SBTE)

Embriology, developmental biology and physiology of reproduction

Developmental rates and quality of blastocysts generated by zinc chelation and intracytoplasmic calcium rise of porcine eggs.

Juan Pedro Cabeza [1], Juan Camera [2], Olinda Briski [1,3], Daniel Salamone [1,3], Andres Gambini [1,2,3]

[1] LABBA - Laboratorio de Biotecnología Animal, Facultad de Agronomía, UBA (Av. San Martin 4453, CABA, Argentina), [2] PEqFAUBA - Producción Equina, Facultad de Agronomía, UBA (Av. San Martin 4453, CABA, Argentina), [3] CONICET - Consejo Nacional de Investigaciones Científicas y Técnicas (Godoy Cruz 2290, CABA, Argentina)

Resumo

Immediately upon the sperm-egg fusion, orchestrated rises of intracytoplasmic calcium (Ca) are followed by the release of zinc (Zn) out to the perivitelline space (zinc sparks). Both elements play an essential role in triggering oocyte activation and embryo development. Our work aimed to determine the optimal condition for porcine egg activation using the Zn chelator 1,10-phenanthroline monohydrate (PHEN, Sigma-Aldrich, CABA,Argentina, Product Number: P9375) and to compare embryo developmental rates and quality of eggs activated with the Ca2+ ionopohre -ionomycin- (IONO), known to increase intracytoplasmic levels of Ca, and consequently induction of the Zn sparks. First, we compared PZM and TALP-H media for zinc chelation at a previously published concentration for pig (0.5mM for 1h; Uh et al., Theriogenology, 125:259-267, 2019; Experiment 1). Afterwards, we determined the optimal concentrations and exposure time for PHEN treatment (Experiment 2). Finally, we compared the effects of Zn chelation before and after ionomycin induction of an intracytoplasmic Ca rise (Experiment 3). Blastocyst quality was determined by immunofluorescence (IF) of SOX2, OCT4 and CDX2. Oocyte collection and IVM were performed as reported (Buemo et. al., PLoS ONE 11(2): e0146390, 2016). Eggs were activated using PHEN or IONO (5mM for 4m in TALP-H). After treatment, zygotes from all experimental groups were incubated for 3h in 1.9mM of 6-Dimethylaminopurine. Embryos were cultured in microdrops of PZM media. Day 7 blastocysts were fixed and subjected to IF analysis using SOX2, OCT4 and CDX2 antibodies (Gambini et al., PLOS ONE 15(9):e0238948, 2020). PHEN in TALP-H resulted in higher blastocyst rates than in PZM (IONO, n=62, 17,74%; PHEN-PZM, n=92, 3,26%; PHEN-TALP-H, n=93, 15,05%) and it was used for experiments 2 and 3. Embryo developmental rates with PHEN 1mM for 30m in TALP-H was higher (n= 85, 44,71%) than IONO (n=97, 20,62%) or other PHEN conditions (PHEN 0.5mM for 1h n=146, 18,49%; PHEN 1mM for 1h n=63, 14,29%). IONO and PHEN blastocysts had similar total cell number (mean ± SEM; n=7, 41.71 ± 3.12 and n=8, 34.13 ± 6.88, respectively) and no significant differences were found in the number of nuclei or expression pattern of the studied markers. Interestingly, Zn chelation after (n=103, 31,07%) or before (n=76, 21,05%) a Ca oscillation impaired blastocyst rate compared to Zn chelation only (n=76, 46,05%) but not with IONO (n=79, 27,85%). In conclusion, we have established new optimal conditions for oocyte activation using PHEN without affecting blastocyst cell number nor the expression of relevant transcription factors, suggesting that Ca oscillations are not essential for their normal in vitro expression in parthenogenetic embryos in pigs. Moreover, the artificial manipulation of both Zn and Ca, in any order, negatively affects embryo development at the concentration tested, possibly for interrupting the orchestrated mechanism needed proper oocyte activation.